COACERVATION/PHASE SEPARATION

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INTRODUCTION

Polymer coacervation is a long established, and widely used, method for reversible gelification and microencapsulation of biological materials such as liquid and solid drug compounds or cells. Coacervation is defined, by IUPAC, as the separation of colloidal systems into two liquid phases. It is to be distinguished from precipitation, which is observed in the form of coagulum or flocs and occurs in colloidally unstable systems (1). The term coacervation was introduced in 1929 by Bungenberg de Jong and Kruyt, for a process in which aqueous colloidal solutions separate, upon alteration of the thermodynamic condition of state, into two liquid phases, one rich in colloid, i.e. the coacervate, and the other containing little colloid (2). Accounting for different phase separation mechanisms, coacervation was subdivided into simple and complex coacervation. In simple coacervation, the polymer is salted out by electrolytes, such as sodium sulfate, or desolvated by the addition of a water miscible nonsolvent such as ethanol, or by an increase or decrease in temperature. Conversely, complex coacervation is essentially driven by the attractive forces of oppositely charged polymers.

The earliest commercial application of coacervation was for the development of "carbonless" carbon copy paper by the National Cash Register Company in the late 1950s (3). More recently, the field of polymer coacervation has developed steadily so that a more refined and complete classification of coacervation systems can be proposed here (Table 1). Other classification schemes and related principles of coacervation for microencapsulation are available in the literature with illustrated examples (4–6).

In this contribution, we discuss coacervation as a phenomenon between one or two polymers in a solvent, although similar phenomena may occur with ionic or highly polarized forms of drugs such as between the anionic heparin and the cationic gentamicin or morphine (4), the anionic surfactants sodium cholate or sodium dodecylsulfate and cationic antidepressants (7), or anionic DNA and cationic forms of gelatin and chitosan in the presence of sulfate ions (8). Further, coacervation-like phenomena are increasingly used to engineer "smart" polymers that undergo reversible strong conformational and macroscopic changes upon small changes in the environment, e.g., pH, temperature, ionic strength (9). These polymers are essentially single or associated polyions designed for stimulus-responsive drug delivery, bioseparation, biomimetic actuators, or materials with switchable hydrophilic/hydrophobic surfaces.

According to the classification proposed in this paper (Table 1), polymer coacervation is generally observed in binary or ternary systems, in either aqueous or organic liquids. Three main mechanisms govern the process of coacervation in these systems: (i) Polymer desolvation, in binary and ternary systems, (ii) Polymer 2–Polymer 3 repulsion in a common solvent of the two dissimilar polymers, i.e., a ternary system; (iii) Poly(ion)–counterion interactions such as between poly(cation) and poly(anion) in a common solvent, i.e., a ternary system; similarly, a poly(H-donor)–poly(H-acceptor) interaction may also lead to polymer coacervation. Thus, polymer coacervation is a direct consequence of changes of molecular interactions operating between polymer–polymer (same species), polymer–solvent, polymer–coacervating agent, or poly(ion)–counterion. Prior to describing the various

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Table 1 Classification of common aqueous and organic systems for polymer coacervation

Binary systems: Coacervation by partial polymer desolvation:	
Solvents (component 1)[a]	Water; Organic solvents
Polymers (P; component 2)[a]	Hydrophilic[b] P^0, P^+, P^-; Lipophilic P
Coacervation inducing factors	Temperature, pH
Ternary systems: Coacervation induced by partial polymer desolvation:	
Solvents (component 1)[a]	Water; Organic solvents
Polymers (P; component 2)[a]	Hydrophilic[b] P^0, P^+, P^-; Lipophilic P
Coacervating agents (component 3)[a]	Nonsolvents for the polymer; Electrolytes ("Simple coacervation")
Ternary systems: Coacervation induced by Polymer 2–Polymer 3 repulsion:	
Solvents (component 1)[a]	Water or organic solvent
Polymers (P2, component 2)[a]	Hydrophilic[b] $P2^0$, $P2^+$, $P2^-$; Lipophilic P2
Coacervating agents (component 3)[a]	Polymer 3, P3
Ternary systems: Coacervation induced by noncovalent polymer cross-linking:	
Solvents (component 1)[a]	Water[c]
Polymers (component 2)[a]	P^+ or P^-
Cross-linking agents (component 3)[a,d]	P^- or P^+ ("Complex coacervation"); Di- and trivalent counter-cations or counter-anions

[a]Numbering of components: 1-designates the solvent for the polymer to be coacervated; 2-designates the polymer to be coacervated; 3-designates the coacervating agent. The numbers are also used as subscripts in the equations.
[b]P^0, P^+, P^- designate nonionic, cationic, and anionic polymers, respectively.
[c]Nonaqueous systems are not described in pharmaceutical technology.
[d]P^- and P^+ or the di- and trivalent counterions are integral components of the coacervate phase.

systems in more detail, it may be helpful to consider first some basic aspects of polymer solution behavior, knowledge that may be useful for understanding coacervation phenomena as well as for optimizing microencapsulation processes based on polymer phase separation.

Polymers dissolved in a solvent are encased in a sheath of solvent molecules that solvate their functional groups, typically through hydrogen-bonding and van der Waals forces. The envelope of solvation prevents chain segments in close proximity from attracting one another by interchain H-bonds, van der Waals or opposite ionic forces. Factors that lower the solvation of dissolved polymers thin out the sheath of solvation so that, at some point, contiguous chains attract one another by secondary valence bonds, thereby forming an entangled network or even noncovalent weak cross-links. Polymer chain desolvation is one type of mechanism leading to phase separation; under certain conditions, gelification rather than phase separation occurs. Factors that lower polymer solvation include temperature change, increase in molecular weight or, for poly(ions), pH-change in binary systems, or the increase in polymer concentration in binary or ternary systems. One very effective way to increase polymer solution concentration is to lower the number of solvent molecules available for polymer solvation. This can, be achieved practically, by adding a third component to the polymer solution (ternary system) such as an electrolyte or a second liquid, which must be a nonsolvent for the polymer. The term nonsolvent is used here for all poor solvents for the polymer to be coacervated. The added electrolyte or nonsolvent will bind part of the polymer solvent. Competition for solvent of solvation will desolvate the polymer molecules leading to phase separation in the form of coacervates or precipitates. When electrolytes are used for polymer desolvation, the phenomenon is called salting-out. In aqueous systems, the effectiveness of dehydration, i.e., a particular form of desolvation, follows the so-called Hofmeister or lyotropic series, which arranges ions in the order of increasing salting-out capacity for hydrocolloids: $NH_4^+ < K^+ < Na^+ < Ca^{2+} < Mg^{2+}$ and $Cl^- <$ acetate$^- < SO_4^{2-} <$ tartrate$^{2-} < HPO_4^{2-} <$ citrate^{3-} (only pharmaceutically acceptable ions are indicated here).

When at least two dissimilar non-ionic, nonpolar, or only slightly polar polymers (Polymer 2 and Polymer 3) are mixed in a common solvent, phase separation generally occurs. This event is thermodynamically controlled and can be explained as follows (10). The dissolution of a polymer in a solvent is commonly endothermic (positive enthalpy of mixing, ΔH_m), thus counteracting dissolution. It is indeed the entropy increase (positive ΔS_m) that allows a polymer to dissolve in a

solvent, i.e., the entropy increases as the arrangement (or lattice) of the solvent molecules are largely disturbed by introducing long polymer chains, which require a relatively large molar volume inside the solution. When a second polymeric species (Polymer 3) is mixed with a solution of Polymer 2 in a common solvent, the two polymer species will typically interact through van der Waals forces, and this interaction is proportional to their molecular weights. This interaction would produce a substantial endothermic energy change whereas the entropy gain by this intermixing is very small owing to the small number of polymer molecules involved. Thus, because of the positive free energy change that would occur if the dissimilar polymers would mix with one another, phase separation into two distinct phases each of them rich in one of the two polymer species is thermodynamically more favorable.

Contrary to the aforementioned mechanism, pairs of oppositely charged poly(ions), or highly polarizable polymers, or of poly(H-donor) and poly(acceptor) tend to interact favorably with one another, i.e., their free interaction energy change is negative owing to a negative ΔH_m. Therefore, they may well coexist in a common solvent, or even attract one another so strongly that the negative ΔH_m dominates over the entropy gain in the common solvent. In this case, the two polymers will form a polymeric complex separating from the solvent. Depending on the strength of enthalpic interaction between the polymeric complex and the solvent, the complex may either precipitate as solid particles or remain partly solvated (complex coacervate). A comparable mechanism operates when a poly(ion) is mixed with a low molecular weight di- or trivalent counterion, such as Ca^{2+}, Mg^{2+}, Al^{3+}, Zn^{2+}, $tartrate^{2-}$. This type of mechanism leads to a strong non-covalent cross-linking of the polymer chains forming a relatively tight network.

Generally, all the mechanisms of polymer coacervation involve some sort of phase separation, thereby producing more or less dense coacervate microdroplets. These microdroplets can either engulf an additional component, such as a dissolved drug, or deposit on solid surfaces, which is typically used for coating solid particles added to the system, e.g., drug particles or living cells.

THERMODYNAMIC MODELS OF POLYMER SOLUBILITY AND PHASE SEPARATION

Fundamental aspects of coacervation have been thoroughly covered for some time through the classical studies of Bungenberg de Jong and Kruyt for ionic systems (2), and by Dobry and Boyer-Kawenoki for non-ionic systems (11). The basic thermodynamic conditions for polymer-solvent interactions and polymer phase separation have been nicely described by Flory (10). In the following, polymer phase separation processes will be briefly considered from mechanistic and thermodynamic points of view.

Classical Models to Describe Coacervation Induced by Temperature Change and Nonsolvent Addition

In the early 1940s, Flory and Huggins proposed, separately, a lattice model to describe polymer solutions and introduced the interaction parameter χ(10). This parameter increases as solvent power decreases; hence, a thermodynamically good solvent is characterized by a low interaction parameter. In practice, most polymer-solvent combinations result in χ-values ranging from 0.2 to 0.6 (12). Moreover, the theory predicts that a polymer will dissolve in a solvent only if the interaction parameter is less than a critical value χ_c, which, at a given temperature, depends on the degree of polymerization (x) of the dissolved polymer (10):

$$\chi_c \approx \frac{1}{2} + \frac{1}{\sqrt{x}} \tag{1}$$

For polymers of very high molecular weight, χ_c approaches the value of 0.5. Upon gradual addition of a nonsolvent (with a large χ value), phase separation occurs in the order of decreasing x, i.e., when $\chi < \chi_c$. Further, χ also depends on temperature. This is generally illustrated by solubility phase diagrams, where single and two-phase systems are defined as a function of polymer volume fraction and temperature. The applicability of χ in coacervation is very limited, as χ is not a constant, but depends on polymer concentration, molecular weight, and temperature. Moreover, χ cannot be determined readily for polymer–liquid pairs and is inconvenient for multi-component systems. Finally, χ is a composite term influenced by hydrogen bonding.

More commonly used descriptors of polymer solubility are the solubility parameters introduced by Hildebrand and Scott (13) for dispersive interaction forces, and extended by Hansen (14) for dispersive (δ_d), polar (δ_p), and hydrogen bonding contributions (δ_h) to interaction energies. An equation sometimes used to estimate the solubility range of Polymer 2 in a solvent (subscript 1) is

$$\delta = [4(\delta_{d1} - \delta_{d2})^2 + (\delta_{p1} - \delta_{p2})^2 + (\delta_{h1} - \delta_{h2})^2]^{0.5} \tag{2}$$

According to Van Krevelen (15), $\Delta\delta$ should not exceed 5 $MPa^{0.5}$ for good solubility. This rule of thumb may be applied to solutions with constant polymer concentration. However, the stepwise addition of a nonsolvent, followed by solvent partitioning between coacervate and continuous liquid, changes continuously the composition of a coacervation dispersion. Under the assumptions that the continuous phase consists exclusively of solvent and nonsolvent, and the coacervate phase of solvent and polymer, the solubility parameters of both phases may be calculated by

$$\delta_{\text{continuous phase}} = \phi_{1,\text{con}}\delta_{\text{solvent}} + \phi_3\delta_{\text{nonsolvent}} \quad (3)$$

$$\delta_{\text{coacervate phase}} = \phi_{1,\text{coa}}\delta_{\text{solvent}} + \phi_2\delta_{\text{Polymer 2}} \quad (4)$$

where $\phi_{1,\text{con}}$, $\phi_{1,\text{coa}}$, ϕ_2, and ϕ_3 are the volume fractions of solvent in the continuous phase, of solvent in the coacervate phase, of polymer, and of nonsolvent, respectively. Although Equations 3 and 4 are empirical mixing equations with limited validity, they are frequently used in pharmaceutical technology to improve solvent mixtures for coating, film formation, and microencapsulation. The mean solubility parameter of the coacervation mixture can be calculated only for a single phase system, i.e., as long as the nonsolvent is soluble in the polymer solution. As solubility parameters of polymers cannot be determined directly from vaporization energy, various calculation methods have been proposed using the cohesive properties of functional groups and assuming additivity of these properties (16). Moreover, polymer solubility experiments in a variety of solvents of known solubility parameters, may be useful to define the borderline of good solvents and nonsolvents for polymer coacervation.

The Interaction Parameter χ to Describe Coacervation Induced by Polymer 2–Polymer 3 Repulsion

The theoretical treatment of polymer phase separation based on polymer-polymer repulsion requires an extension of the χ-parameter concept on two polymers in a common solvent. For this case, Scott (17) defined the critical conditions for phase separation, provided that the rather common conditions apply that $|\chi_{1,2} - \chi_{1,3}| \ll 1$ and $\sqrt{x_2} < \sqrt{x_3} < x_2 \ldots$

$$(\chi_c)_{2,3} = 0.5\left(\frac{1}{\sqrt{x_2}} + \frac{1}{\sqrt{x_3}}\right)^2\left(\frac{1}{1-\phi_1}\right) \quad (5)$$

where the subscripts 1, 2, and 3 refer to the solvent, the polymer to be coacervated (Polymer 2), and the second polymer (Polymer 3; coacervating agent). If the Polymer 2 – Polymer 3 interaction parameter $\chi_{2,3}$ is approximated from solubility parameters (Eq. 6), estimated experimentally or calculated from group contributions

$$\chi \approx \frac{V_1}{RT}(\delta_2 - \delta_3)^2 \quad (6)$$

then, the critical volume fractions of the solvent (ϕ_1) and of both polymers (ϕ_2, ϕ_3) can be calculated from

$$\frac{\phi_2}{1-\phi_1} = \frac{\sqrt{x_2}}{\sqrt{x_2} + \sqrt{x_3}} \quad (7)$$

$$\frac{\phi_3}{1-\phi_1} = \frac{\sqrt{x_3}}{\sqrt{x_2} + \sqrt{x_3}} \quad (8)$$

Unless the solubility parameters of both polymers are very similar, the polymers will be incompatible, leading to polymer phase separation, as $\chi_{2,3} > (\chi_c)_{2,3}$.

New Approaches to Characterize Polymer Coacervation

Another interesting approach to describe polymer coacervation is that of Van Oss (18), who defined the conditions for simple and complex coacervation by the interfacial interactions between similar and dissimilar molecules. In this model, the solubility, s, of the Polymer 2 in a solvent (subscript 1) depends on the free energy of interfacial interaction, ΔG_{212}:

$$RT \ln s = f(G_{212}^{\text{IF}}) \quad (9)$$

According to this equation, the solubility of the polymer molecules in a liquid will increase with increasing positive values of $\Delta G_{212}^{\text{IF}}$, as the polymer molecules will increasingly repel each other and thereby tend to disperse. If $\Delta G_{212}^{\text{IF}} < 0$, solubility decreases due to molecular attraction. In most pharmaceutical systems, molecular contributions to $\Delta G_{212}^{\text{IF}}$ will be from apolar Lifshitz-van der Waals ($\Delta G_{212}^{\text{LW}}$) forces and from Lewis acid/base ($\Delta G_{212}^{\text{AB}}$) forces. Hence, $\Delta G_{212}^{\text{IF}}$ is represented by

$$G_{212}^{\text{IF}} = -2\left(\sqrt{\gamma_2^{\text{LW}}} - \sqrt{\gamma_1^{\text{LW}}}\right)^2 - 4\left(\sqrt{\gamma_2^+\gamma_2^-} + \sqrt{\gamma_1^+\gamma_1^-} - \sqrt{\gamma_2^+\gamma_1^-} - \sqrt{\gamma_2^-\gamma_1^+}\right) \quad (10)$$

where the second part of the right hand side describes the Lewis acid/base interactions. For a ternary system with two dissimilar Polymer 2 and Polymer 3 immersed in a solvent 1, $\Delta G_{212}^{\text{IF}}$ becomes

$$G_{213}^{IF} = \left(\sqrt{\gamma_2^{LW}} - \sqrt{\gamma_3^{LW}}\right)^2 - \left(\sqrt{\gamma_2^{LW}} - \sqrt{\gamma_1^{LW}}\right)^2 - \left(\sqrt{\gamma_3^{LW}} - \sqrt{\gamma_1^{LW}}\right)^2 + 2\sqrt{\gamma_1^+}\left(\sqrt{\gamma_2^-} + \sqrt{\gamma_3^-} - \sqrt{\gamma_1^+}\right) + 2\sqrt{\gamma_1^-}\left(\sqrt{\gamma_2^+} + \sqrt{\gamma_3^+} - \sqrt{\gamma_1^+}\right) - 2\sqrt{\gamma_2^+\gamma_3^-} - \sqrt{\gamma_2^-\gamma_3^+} \quad (11)$$

For all exclusive Van der Waals interactions, the interfacial interaction energy between Polymer 2 and Polymer 3 immersed in a liquid reduces to

$$G_{213}^{LW} = \gamma_{23}^{LW} - \gamma_{21}^{LW} - \gamma_{31}^{LW} \quad (12)$$

which can be rewritten as

$$G_{213}^{LW} = \left(\sqrt{\gamma_2^{LW}} - \sqrt{\gamma_1^{LW}}\right)\left(\sqrt{\gamma_1^{LW}} - \sqrt{\gamma_3^{LW}}\right) \quad (13)$$

In coacervation processes induced by Polymer 2–Polymer 3 repulsion, the conditions for coacervation are, $\Delta G_{212}^{IF} > 0$. In apolar systems, this condition is fulfilled by $\gamma_2^{LW} > \gamma_1^{LW} > \gamma_3^{LW}$ or by $\gamma_2^{LW} < \gamma_1^{LW} < \gamma_3^{LW}$. In polar systems, however, coacervation becomes only predictable by solving Eq. 11. Contrary to simple coacervation, complex coacervation in a ternary system occurs only if the polymer molecules attract each other, and hence, the interfacial free energy becomes negative.

The apparent advantage of the Van Oss theory lies in the accessibility of the surface tension parameters. For a given solute, γ_2 can be determined from contact angle and Young's equation. Moreover, these parameters can also be derived theoretically from polymer group contributions as described by Van Krevelen (15). On the other side, it remains questionable if the Van Oss model is valid for systems where hydrogen bonding plays an important role, as interfacial tension measurements are unlikely to account for H-bonding forces.

POLYMER COACERVATION INDUCED BY PARTIAL POLYMER DESOLVATION (SIMPLE COACERVATION)

Process Description

Simple polymer coacervation is based on partial polymer desolvation in binary or ternary systems. This partial polymer desolvation may be induced by changing the temperature of the polymer solution, by adding to the polymer solution a poor solvent (or nonsolvent) for the polymer, or by "salting-out" by electrolytes. The coacervate phase may form droplets in the stirred equilibrium phase or deposit as a film on a given solid or liquid surface, such as solid drug particles or droplets of aqueous drug solutions. In both situations, the coacervate can be stabilized by intermolecular physical or covalent cross-linking, which typically can be achieved by altering the pH or the temperature, or by adding a cross-linking agent (19). In pharmaceutical technology, simple coacervation, alike other coacervation types, is very frequently used to entrap drugs into microcapsules or micromatrices; the term microspheres will be used hereafter for both types of microparticles. If a drug should be entrapped in the coacervated polymer, a liquid or solid form of the drug is dissolved or dispersed in the polymer solution. Here, the principles of simple coacervation will be illustrated for two frequently used polymer types.

A very commonly employed simple coacervation procedure utilizes gelatin. Simple coacervation of gelatin typically involves the use of water-miscible nonsolvents for gelatin, such as alcohols, or salts, such as sodium sulfate. This produces a partial dehydration of the gelatin molecules at a temperature above the gelling point and leads to separation of a liquid gelatin-rich phase and an equilibrium liquid containing only minor amounts of gelatin. (Fig. 1). Finally, the coacervate droplets in the equilibrium liquid are hardened by physical or covalent cross-links (for example, by adjusting the pH to the isoelectric point or adding glutaraldehyde or formaldehyde).

A very frequently described family of polymers subjected to simple coacervation are cellulose derivatives, particularly ethyl cellulose (EC) (20). While most cellulose ethers are soluble in water, EC and the cellulose esters are insoluble or only partly soluble in water, e.g., as a function of pH. For coacervation of EC, toluene is a preferred good solvent and cyclohexane a poor solvent (21–23). Gradual addition of cyclohexane to a solution of EC desolvates the polymer. Alternatively, EC can be dissolved in hot cyclohexane; cooling to room temperature induces polymer phase separation. In both these cases, the coacervate film or droplets can be hardened by exposing the coacervate to a large volume of cyclohexane, whereby physical cross-links are formed.

Materials

Gelatin (24, 25) and cellulose derivatives (26, 27) are probably the most widely used polymers in simple coacervation for pharmaceutical purposes, although various other polymers have been successfully employed for the production of microcapsules by this process. In principle, any polymer can be utilized as a wall-forming material, provided that partial desolvation can be achieved.

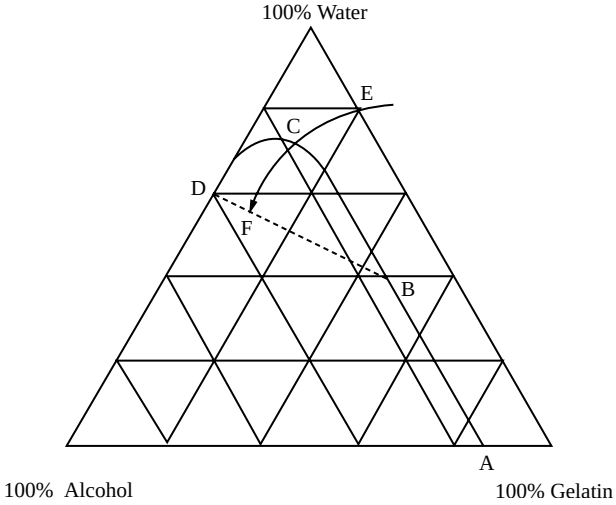

Fig. 1 Schematic ternary diagram of a gelatin–water–alcohol system. The diagram shows the zone where two phases exist, i.e., the coacervate and equilibrium phases. The two-phase systems are represented by the area under the binodial curve (points A, B, C, D), whereas the area above the binodial represents single liquid phase systems of gelatin dissolved in water–alcohol mixtures. The arrow (points E, C, F) indicates the addition of alcohol into an aqueous solution of gelatin at an initial concentration of 20% gelatin. At approx. 18% alcohol (point C), phase separation occurs. At a given composition (point F), the two phases have the compositions denoted by the points D (equilibrium phase consisting of 40% alcohol, and 60% water) and B (coacervate phase consisting of approx. 46% gelatin, 15% alcohol and 39% water) situated at both sides of the so-called tie-line (dotted line). The ratio of coacervate to equilibrium phases, on a weight basis, is given by the ratio D-F/F-B.

The polymers subjected to simple coacervation include, amongst others, poly(styrene), poly(vinyl acetate), poly(vinyl chloride), poly(lactide) (5), poly(vinyl alcohol) (28), poly(acrylates) (29), chitosan/PVA-blends (30, 31), albumin (32), casein (33), and also vegetable proteins like vicilin (34), and legumin (35). For more detailed information, the reader is advised to consult the extensive reviews in this field (5, 6, 36, 37).

For simple coacervation induced by nonsolvent addition in aqueous systems, ethanol, acetone, dioxane, isopropanol, and propanol are the most preferred to cause polymer desolvation and phase separation. In organic systems, mainly nonpolar solvents, such as petroleum ether, cyclohexane, and paraffin, are useful.

Critical Process Steps and Product Characteristics

Simple coacervation involves four distinct steps: i) Phase separation upon polymer desolvation; ii) Droplet formation or deposition of the coacervate phase on a given surface; iii) Hardening of the coacervate phase; and iv) Isolation of microparticles or surface-coated material. Coacervation is quite a complex physico-chemical phenomenon, and many factors affect the properties of the resulting product. Simple coacervation in organic and aqueous systems depends on molecular interactions of the materials involved at a given temperature, the presence of solid or liquid surfaces with a high affinity for the coacervate phase, the rate of polymer desolvation and fluid dynamic processes, e.g., diffusion, laminar or turbulent movements. Therefore, for any given system, the material and process parameters must be carefully studied to control fully the process. In general, simple coacervation is a relatively slow process where diffusion and partitioning of the components between separated phases may take a considerable length of time to reach equilibrium. Therefore, the process is commonly performed under non-equilibrium conditions. This makes the rate and duration of every process step very critical for obtaining reproducible products and preventing undesired coalescence or precipitation. All these difficulties, along with the need for sometimes large amounts of organic solvents and toxicologically critical cross-linking agents have probably hampered the introduction of this process into an industrial setting.

Applications

To our knowledge, simple coacervation has essentially remained a technology described by academics and used for research rather than in pharmaceutical industry. Green (38) first demonstrated the microencapsulation of oil droplets by simple coacervation of gelatin. In this study, gelatin coacervation was induced by sodium or ammonium sulfate. Since then, simple coacervation has been used to encapsulate foods, flavors, and pharmaceuticals (19).

Simple coacervation of cellulose derivatives has been used for microencapsulation of various drugs, such as theophylline (27), ibuprofen (39), indomethacin (26), adryamicin (40), and nicardipine (41). The goal of microencapsulating these drugs was to decrease their gastric irritation, mask the bitter taste and, very importantly, to achieve sustained release.

POLYMER COACERVATION INDUCED BY POLYMER 2–POLYMER 3 REPULSION IN TERNARY SYSTEMS

Process Description

In coacervation by Polymer 2–Polymer 3 repulsion, the addition of Polymer 3 causes phase separation between the

two polymer species dissolved in a common solvent 1. This phase separation produces a viscous, liquid phase of Polymer 2, i.e., the coacervate, and a low-viscous phase of Polymer 3, often called continuous or polymer-poor phase. Under stirring, coacervate droplets are formed and dispersed in the continuous phase. The solubility of Polymer 3 in solvent 1 should be superior to that of Polymer 2 in this common solvent. For particle production, the Polymer 3 should also function as stabilizer for the coacervate droplets to prevent their aggregation. Further, for the entrapment of a biologically active material, the coacervate must have a certain degree of fluidity and a high affinity to the core material, whereas the affinity between core material and continuous phase should be low (see below). Finally, the coacervate droplets still contain a substantial amount of solvent 1 and are, therefore, too soft to isolate. Hardening of the coacervate droplets by a hardening agent is required before the product can be collected as discrete microspheres or microcapsules. A typical experimental set-up for this coacervation process yielding polymeric particles is illustrated in Fig. 2.

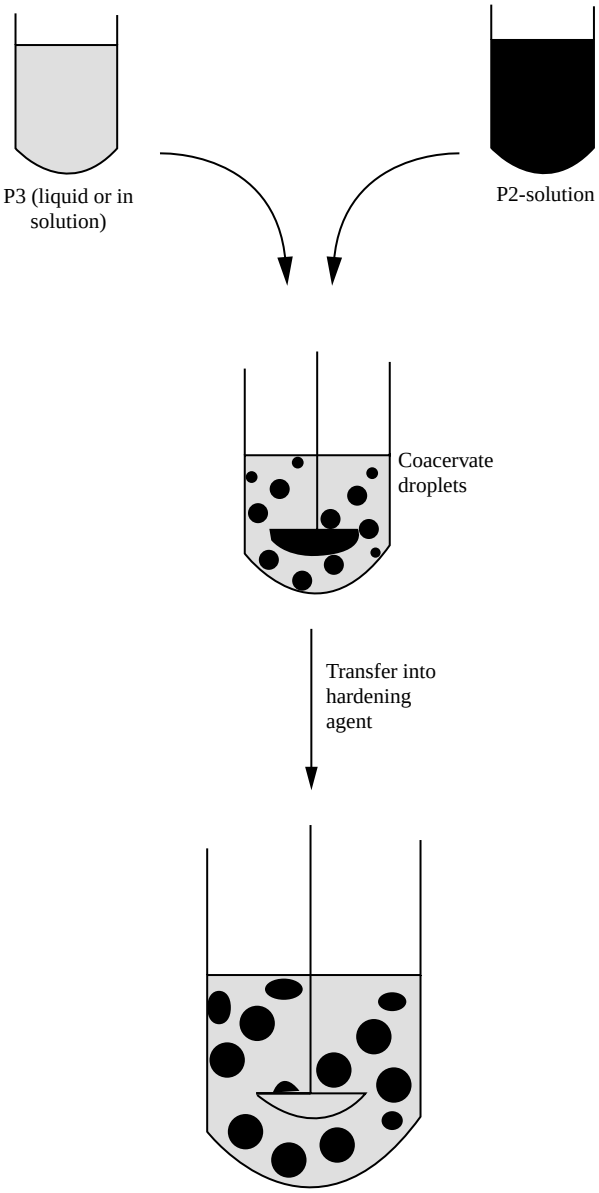

Fig. 2 Experimental set-up of a typical coacervation process by Polymer 2–Polymer 3 repulsion.

Materials

Coacervation by Polymer 2–Polymer 3 repulsion has been applied to various polymers, although the biodegradable poly(lactide) (PLA) and poly(lactide-co-glycolides) (PLGA) have attracted the highest interest, particularly for drug microencapsulation and controlled drug delivery (42). PLA/PLGA are commonly dissolved in dichloromethane or ethyl acetate and coacervated by adding a second, relatively low molecular weight polymer such as silicone oil (poly(dimethyl siloxane), PDMS, 100–1000 mPas). For hardening the coacervate droplets, the coacervate mixture is transferred into a nonsolvent for Polymer 2, such as hexane, heptane, or low molecular weight cyclic siloxanes, e.g., octamethylcyclotetrasiloxane (OMCTS) or decamethylcyclopentosiloxane (DMCPS).

To a very limited extent, other polymers have been coacervated by this process to form microparticles, i.e., ethylcellulose, cellulose nitrate, poly(methyl methacrylate), cellulose acetate phthalate, poly(acrylonitrile-co-styrene), and poly(styrene) (4). For some of these, liquid poly(butadiene) (8–10 kDa) was employed as a coacervating agent.

The hardening of coacervate droplets may be accomplished either by subsequent desolvation and formation of van der Waals bonds or covalent cross-linking of the coacervated polymer (4). In the first process, the coacervation mixture is transferred into a liquid which is a nonsolvent for Polymer 2, though it is a good solvent for Polymer 3. Examples of such hardening agents include hexane, heptane, very low molecular weight volatile siloxanes (OMCTS, DMCPS). In the chemical hardening process, a cross-linking agent, e.g., glutaraldehyde or diisocyantes, is added to the coacervate system. Typical functional groups on Polymer 2 that are useful for cross-linking reactions are hydroxyl, e.g., in cellulose ethers and esters, and amine groups.

Naturally, the aforementioned polymers may also be coacervated by desolvation upon adding a nonsolvent, such as hexane, heptane, liquid paraffin, or a vegetable oil. The advantage of using the coacervation by Polymer 2–Polymer 3 repulsion is that the viscosity and volume fraction of the coacervate phase and the stability of coacervated droplets can be controlled by the amount of added Polymer 3 (43). The control of these coacervate characteristics is important for preventing aggregation of coacervate droplets and for efficient microencapsulation of biologically active materials.

Critical Process Steps and Product Characteristics

The particular feature of coacervation by Polymer 2–Polymer 3 repulsion is that phase separation occurs already after the addition of a minute volume fraction of Polymer 3, which is in contrast to the coacervation by polymer desolvation (44). In the very first step, a dispersion of Polymer 3-in-Polymer 2 phase is formed (Fig. 3). Further Polymer 3 addition produces a phase inversion, whereupon the Polymer 2 phase (coacervate droplets) is dispersed in the Polymer 3 phase. Upon further Polymer 3 addition, the solvent is partially extracted from the coacervate droplets thereby increasing their viscosity and physical stability against coalescence. Optimal coacervate stability is generally achieved within a certain range of Polymer 3 volume fraction. This "stability window" has been determined by various authors for different PLA and PLGA types (43).

The main advantage of coacervation by Polymer 2–Polymer 3 repulsion over polymer desolvation resides in the good control of the composition and viscosity of both the coacervate and dispersing phases. This, in turn, provides a means to control particle size and prevent undesired coalescence of the coacervate droplets, as well as a way to enhance the wetting and engulfing of any biologically active material in either solid or liquid form. Indeed, encapsulation of core material requires primarily that the work of adhesion between core material and coacervate is substantially higher than that between core material and dispersing phase (44), which again depends on the respective composition of the two phases. Further, a certain degree of fluidity of the coacervate droplets will also increase qualitatively and quantitatively the encapsulation process.

Studies dedicated to drug microencapsulation by this type of coacervation method revealed that this method is quite suitable for the microencapsulation of peptide and protein drugs into PLA and PLGA in terms of encapsulation efficiency. However, its drawback lies in the residual coacervating and hardening agents remaining in the microparticles that cannot be eliminated sufficiently and may hamper biomedical use. Therefore, efforts have been made to use coacervating and hardening agents that are relatively safe and to minimize the residues of processing liquids in the final product (45).

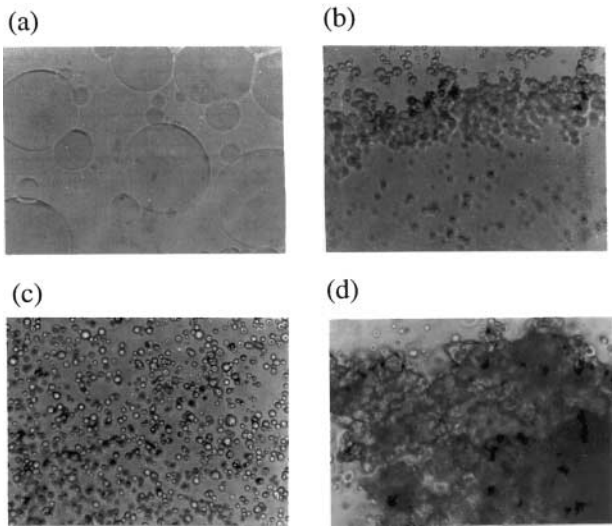

Fig. 3 Different stages of PLA coacervation by PLA–silicone oil (PDMS) repulsion in a ternary system using dichloromethane (DCM) as solvent: (a) Dispersion of PDMS in PLA/DCM; (b) Phase inversion yielding unstable droplets of PLA/DCM in PDMS; (c) Stable dispersion of well defined coacervate droplets; (d) Aggregation and precipitation of polymer particles upon exceeding PDMS-addition.

Applications

Coacervation by Polymer 2–Polymer 3 repulsion has been widely used in the field of drug microencapsulation into the biodegradable PLA and PLGA. (42) (see also the chapter on *Microsphere Technology and Applications* of this Encyclopedia). This type of microspheres is intended for parenteral administration and controlled delivery of low doses of very potent drugs. The microparticles produced by this method lie within a size range of 20–150 μm, depending on the process parameters, and can be injected by a conventional syringe and needle. To our knowledge, there is at least one PLGA-based microsphere product on the market for parenteral use that is produced by this particular method: Decapeptyl® Retard (Ferring AG, Kiel, Germany), which contains the LHRH-analog decapeptide triptorelin.

POLYMER COACERVATION UPON NONCOVALENT POLYMER CROSS-LINKING IN TERNARY SYSTEMS

Principles and Mechanisms

Complex coacervation in aqueous solution may be considered as a special case of network formation. Intermolecular forces such as Coulomb, van der Waals, hydrophobic, hydrogen bond, and dipol-charge transfer between polymers themselves, or polymers and low molar mass counterparts, cause phase separation, where the polymers are concentrated in a gel-like phase or a precipitate. The quality of the separated phases strongly depends on the chemical nature of the participating molecules and the separation conditions. The following combinations based on various dominating interactions have been reported:

1. Oppositely charged polyelectrolytes ("polysalt")
2. Highly polarizable polymers
3. H-donor/acceptor polymers
4. Polyelectrolytes/multivalent counterions

Fig. 4 illustrates the complex coacervation for systems 1 and 4. In system one, the complex is composed of two different polymer structures intermolecularly cross-linked (Fig. 4a), whereas in system 2, the complex is formed by the inter- and/or intramolecular bridging of a single polymer structure (Fig. 4b). Specific examples for the two complex types are sodium alginate (polyanion) with chitosan (polycation), and sodium alginate (polyanion)/ calcium (divalent counterion), respectively.

From a kinetic point of view, the complex formation between a cationic and an anionic site is generally rapid, with rates in the order of fractions of seconds, though hours and days are occasionally required for the total process (46). The rate-determining steps for complex formation are

- Diffusion processes for intermolecular contacts
- Rearrangements of the complex coacervate including both conformational changes and disentanglements.

In particular, these arrangements are relevant for polyelectrolyte components of low charge density, and/or polyelectrolyte components largely differing in molar mass, and concentrated systems (47).

The stoichiometry between polyelectrolytes has very often found to be 1:1 (48), though the optimal ratio of polyions can be as high as 20:1. Generally, as the polymer concentration decreases, the number of chains participating in the complex rises. Furthermore, the charge density required for coacervation is lower if the polymer-solvent interaction parameter (Flory–Huggins) is higher. Strong polyelectrolytes generally participate in 1:1 stoichiometries, though steric hindrance, for example via long pendant groups such as are typical in synthetic quaternary ammoniums, can disturb the so-called symplex ratio (49).

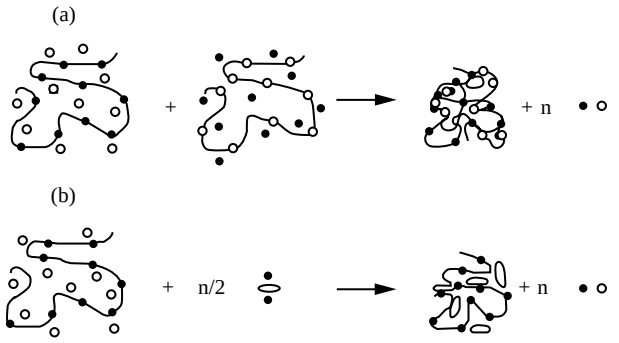

Fig. 4 Principles of complex aqueous coacervation: (a) Oppositely charged polyelectrolytes; (b) polyelectrolyte/ multivalent counterions.

Macromolecular Characteristics for Effective Polymer–Polymer Coacervates

For both polymer-polymer complexation via ionic interactions and hydrogen bonding there is a critical chain length below which competitive binding is impossible (50). In general, polysalt formation is a function of various parameters including molar mass/chain length, charge density, chemical structure, type of the ionic group, and chain architecture. Interestingly, two polyelectrolytes of the same net charge can be complexed provided one is ampholytic, such as gelatin, and is polarized in the electric field of the other (51). In addition, medium conditions (pH, ionic strength, total concentration, temperature) influence the complex formation kinetics and complex properties.

Alginate-poly(L-lysine) and alginate-chitosan are, by far, the most common polymer-polymer coacervation systems, with the former often pregelled, in bead form, with divalent cations such as calcium or barium. As Table 2 indicates, polysaccharides with a rigid structure are generally favored for polysalt formation.

The polyanion–polycation combination generally involves one permanently charged polymer with a second whose charge density is pH-sensitive. Secondary interactions, principally hydrogen bonding, are usually required as is the flexibility of one of the chains (52).

For alginates, the copolymer composition (ratio of mannuronic to guluronic acid units) can influence the

Table 2 Typical polymers employed in complex aqueous coacervation

Polyanions	Polycations
Alginate	Chitosan
Carrageenan	Poly(diallyldimethylammonium chloride)
Carboxymethylcellulose	Poly(L-lysine)
Chondroitin sulfate	Poly(vinylamine)
Cellulose sulfate	
Gellan	
Hyaluronic acid	
Poly(acrylic acid)	
Xanthan	

ultimate complex properties. These include elasticity as well as permeability and mechanical resistance of coacervates cast into 2D or spherical membrane structures. The type of polymer-polymer coacervate (precipitate, sol, network) will also often be highly molar mass dependent, with useful membranes found within a narrow window. This often does not correspond to the molar mass range required for bioapplications, which is dictated by factors such as cell toxicity and biocompatability.

Characterization

The most important applications of polymer-polymer coacervates involve the formation of semipermeable membranes. They represent ionically cross-linked polymer network structures and are generally permeable to water and low molar mass solutes, though they block macromolecules and proteins above a certain molar mass. Molar mass cut-off (MMCO) experiments are carried out, for example, via inverse-size exclusion chromatography. These nontrivial experiments (53) monitor the ingress of a synthetic probe or the egress of biomacromolecules. The complex steric and electrostatic interactions involved in passing through the tortuous pores imply that the apparent MMCO is dependent on the chemistry of the macrosolute. In particular, a higher cross-linking density results in a lower MMCO, though this is rigorously true only if the network is inert, which is frequently not the case (54). Furthermore, most polyelectrolyte complex gels require characterization via transport studies (kinetics) as well as equilibrium diffusion measures (thermodynamics).

Polymer-polymer coacervates, in microcapsule form, can be characterized by a number of methods, though the most accurate involves a compression-based micromanipulated probe connected to a sensitive transducer (55). The precision of such techniques can be as high as 10%. Generally, a 1:1 stoichiometry provides the most stable microcapsules. Other properties, including the sphericity, transparency, or membrane homogeneity are also often characterized. Novel methods based on analytical ultracentrifugation are particularly useful for 2D membranes (56).

Applications

Table 3 lists some of the typical polymer-polymer coacervation systems investigated for microcapsule formation.

The system sodium cellulose sulfate/PDADMAC permits the encapsulation of sensitive biological materials by a simple one step procedure. The broad variety of encapsulation problems so far successfully solved by this system includes the encapsulation of biocatalysts (57, 58), hepatic microsomes for extracorporal detoxification (59), cattle embryos (47), and various drugs for targeted or controlled-release delivery (60).

Polysalts have been prepared as microcapsules for enzyme entrapment (biocatalysts), in the separation of proteins (60) as well as in surfactant binding. Protein separation is particularly selective, with biomacromolecules separated according to their isoelectric point. Coacervation is the preferred method for water soluble drugs where no modification of the absorption kinetics are warranted.

Coacervation for Cell Encapsulation

Processing

Cell encapsulation by polymer-polymer coacervation is generally performed by extrusion of a polyanion solution, seeded with the cells of interest, into a collecting batch containing a cationic simple multivalent electrolyte and/or polyelectrolyte. As an alternative method, the emulsification of polymer-cell suspension in a vegetable oil followed by the internal gelation of the polyanion has been described (62). The latter results in more homogeneous gels. Cell microencapsulation is complex (63) and involves the following steps:

- Polymer sterilization (thermal; UV- or γ-radiation; chemical, e.g., ethylene oxide)
- Polymer depyrogenation to destroy or remove potentially toxic lipopolysaccharides
- Polymer dissolution, often in culture media, to a controlled solution viscosity
- Solution dilution under sterile conditions

Table 3 Typical polymer–polymer coacervates

Polyanion	Polycation	Reference
Alginate (sodium)	Poly(L-lysine)	(76)
	Chitosan	(77)
Cellulose sulfate (sodium)	Poly(diallyldimethylammonium-chloride) (PDADMAC)	(60)
κ-Carrageenan	DEAE-dextran	(78)
Polyphosphate (sodium)	Chitosan	(79)
Chondroitin sulfate (sodium)	Collagen	(80)
	Gelatin	(81)
Dextran sulfate	Ionene	(82)
Poly(acrylic acid)	Poly(ethyleneimine)	(83)
Gelatin	Acacia	(84)
	Arabic gum	(85)
	Chitosan	(86)

- Coacervation reaction control (polyion concentrations, contact time)
- Washing, required to remove nonreacted excess polymer
- Coating to reduce permeability and/or modify surface properties

Examples of cell encapsulation

Fig. 5 shows microencapsulated mice islets intended for intraportal (liver) transplantation to achieve clinical normoglycemia. The islet's β-cells produce insulin in response to a blood glucose stimulus providing a therapeutic alternative to daily insulin injections. The capsule size is optimized to permit oxygen diffusion (64) given an O_2-diffusion distance of 0.2 mm in hydrogels. The specific chemistry permits the simultaneous control of permeability and mechanical properties. From extended studies it has been concluded that multicomponent polymer systems can offer advantages in comparison to binary systems (65), with the thick membrane acting as an entrapment zone and permitting the de-coupling of diffusive and mechanical characteristics.

The encapsulation of hepatocytes for a bioartificial liver, and cell therapy for the treatment of other hormone deficiencies or neurodegenerative diseases, such as Alzheimer's and Parkinson's, are also under investigation. Additional examples of cell encapsulation in polymer-polymer coacervates include non-autologous gene therapy (66), blood substitutes (67) as well as the treatment of prostate cancer (68). Pharmaceutical applications of microcapsules encompass, in addition, transdermal drug delivery and protein delivery such as is required in anti-inflammatory therapy for arthritis.

Scale-Up

While it is not the purpose of this chapter to produce an extensive list of technologies related to polyelectrolyte complexes, some unique examples warrant discussion. Shioya (69) has patented an encapsulation method that controls permeability, while Dautzenberg disclosed a general method, and family of materials, for polyelectrolyte complexation (70). Recently, Vorlop has developed a mechanical cutting method to divide a fluid stream and achieve throughputs as high as 10^4 kg/h (71). Other techniques useful for high volume applications include the rotating disk atomizer.

Applications in Analytics

Polymer-polymer complexation is generally detected via conductometric or potentiometric titrations. "Colloid titration" represents an inverse-system where a polymer with known characteristics, such as potassium poly (vinylalcohol-sulfate) or poly(diallyldimethylammoniumchloride), are used to quantify the concentration of polycation or polyanion, hence relying on a 1:1 stoichiometry (47). Using the cationic dye, toluidine blue, as an indicator, a metachromatic end point is detected. Both methods are volumetric.

INDUSTRIAL VIEW OF THE USEFULNESS OF COACERVATION AND RELATED PRODUCTS

Various microencapsulation techniques have been successfully applied for several years in industry, with a large number of patent applications filed to protect market

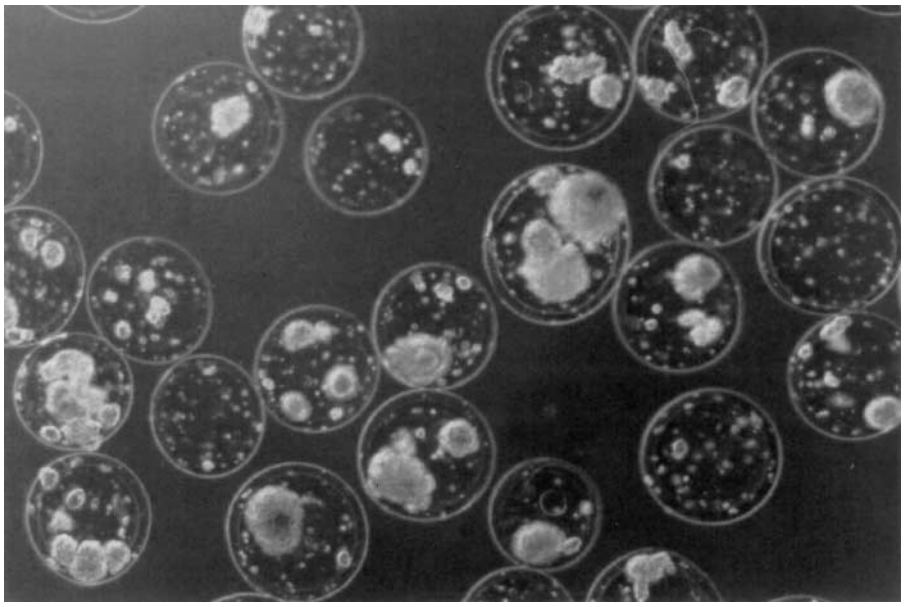

Fig. 5 Encapsulated mice islets in a 400 μm capsules made from sodium alginate/sodium cellulose sulfate//poly(methylene-*co*-guanidine) hydrochloride/ $CaCl_2$.

shares and products, particularly in the area of contrast agents for diagnostic imaging, in agriculture, and in the food industry. In contrast to the many available consumer products based on encapsulation technology, only very few pharmaceuticals have succeeded in the market.

Primarily for safety reasons, manufacturing and marketing of pharmaceuticals is strongly controlled by regulatory guidelines, making product development and introduction especially challenging. Clearly, industrial large scale application of a technology such as coacervation primarily requires a sound and robust process and product design, which is the starting point of all full development work, but is also driven by other important criteria as outlined in Fig. 6. How do these basic considerations actually apply to products prepared by coacervation? To answer this question, the process of organic phase separation for preparing sustained release microspheres for parenteral administration is considered here as an example to illustrate the implications.

Key parameters for the quality of controlled release microspheres for parenteral use encompass the drug encapsulation efficiency, stability over time, syringeability, reproducibility of release kinetics, sterility, therapeutic efficacy, general safety, and local tolerability. Some of these aspects apply to the products made by all microencapsulation techniques, whereas others are mainly relevant to formulations prepared by coacervation. Furthermore for injection or aerosolizing, microspheres require a compatible dispersant to wet the particles and keep them suspended for administration. The reconstitution vehicle will require developmental efforts as the surface properties of microspheres may be hydropobic, especially when the particles have been prepared by coacervation and subsequent physical hardening in lipophilic hardening agent. From the authors' experience, insufficient attention is sometimes given to the development of the dispersant formulation, especially if

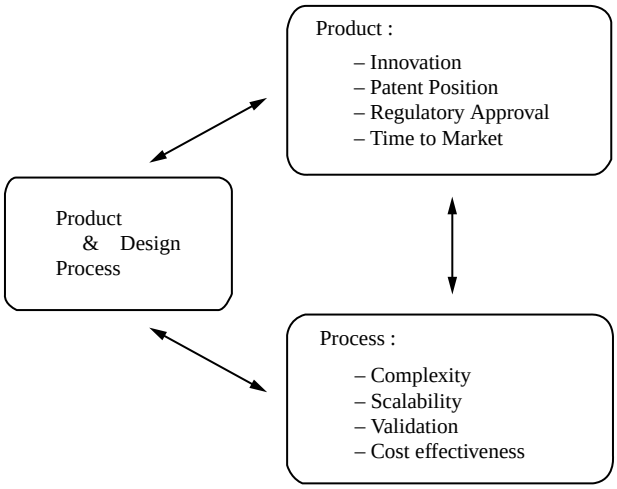

Fig. 6 Key elements for successful application of coacervation in the pharmaceutical industry.

active material and diluent are not manufactured at the same site.

Coacervation is a typical batch process requiring standard equipment with little need for costly investment, which contrasts to continuous microencapsulation processes such as aseptic spray-drying. For scalability, adequate and reproducible shear and stirring conditions, irrespective of the batch size, are very important. Selection of lab and pilot equipments suitable for scaling-up and featured with the necessary instruments to monitor critical parameters such as stirring and temperature are the basis for developmental work. Moreover, the introduction and dispersion of a given drug or bioactive material in the coacervation system also deserves particular attention. The particle size and dispersability of drug powder or drug solution are key parameters for high and consistent encapsulation efficiency and reproducible release kinetics. In organic phase separation, cleaning validation is a very challenging task, as coacervating and hardening agents require cleaning procedures with solvents and detergents suitable to remove efficaciously all coacervation components including non-entrapped drug or bioactive material. Because of this difficulty, the equipment of such critical processes are often product-dedicated to avoid any cross-contamination. In this respect, microencapsulation in aqueous systems is much easier to handle than in organic systems.

Nonetheless, organic phase separation remains an attractive method to encapsulate highly active, water soluble drugs such as peptides and proteins. The involvement of large quantities of organic solvents for production has, however, implications for the design of explosion-proof equipment and facilities. Further, residual solvents and hardening agents may hamper significant achievements for safety concerns. Efforts have therefore been devoted to replace the commonly used hydrocarbons or siloxanes by more biocompatible hardening agents such as isoproyl-myristate or propyleneglycol-octanoate/decanoate, which is used for the hardening of commercial PLGA microspheres containing triptoreline (Decapeptyl(®) Retard; Ferring). Evidently, such improvements are also relevant for patent protection (72, 73). Importantly, changes in processing solvents do not necessitate a new drug file application, but will be treated by the regulatory authorities as a postapproval change of an already approved process. Nowadays, where time to market is the key parameter for success, the choice of well characterized and widely accepted materials is of crucial importance. In this light, coacervation is a demanding technology because the number of useful and safe polymers, solvents and hardening agents is limited. Consequently, the quality, availability, and cost of materials requires careful evaluation before decisions are made to introduce this technology in a pharmaceutical industry. Naturally, such a decision must also be driven by the therapeutic needs. Life saving indications, such as cancer treatment, may profit from a fast track approval by the authorities who balance carefully the pros and cons of possible formulation drawbacks and expected therapeutic benefit. Not surprisingly, microencapsulated parenteral depot formulations are found mainly in niche indications, where a limited and well controlled number of administrations in a relatively small group of patients is required.

Although coacervation is successfully used in pharmaceutical industry, the complexity of this and other microencapsulation technologies must not be underestimated, particularly in view of large scale manufacturing under aseptic conditions. Process design must consider this already at the developmental stage by keeping the number of individual manufacturing steps minimal. In this respect, sterile products prepared by organic phase separation require a significant number of unit operations:

- Sterile filtration/sterilization of excipients
- Mixing/dispersing and stirring
- Hardening by physical or chemical means
- Filtration
- Washing and sieving
- Dispensing and primary packaging (vials, syringes)
- Freeze-drying
- Terminal sterilization or full aseptic processing

For stability and safety reasons, full aseptic processing in a clean room environment is still the method of choice, although the intense involvement of personnel for a aseptic coacervation processing bears a substantial risk of contamination. By contrast, terminal sterilization by gamma or electron radiation provides a very high level of sterility assurance, but the efforts for validation and documentation of radiation sterilization are significant (74, 75). Moreover, radiation sterilization, although standard in the medical device industry, is not yet well accepted in all countries, a fact which again may hamper multinational filing and delay launch of a product.

In summary, the feasibility of coacervation has been shown on industrial scale, although it is probably fair to say that this technology is only scarcely used in pharmaceutical industry. Primarily for its physicochemical complexity and limitations with respect to residuals or processing components, efforts have been made to replace the coacervation technique by simpler technologies. To what extend new applications of coacervation technology in pharmaceutical industry will be successfully

commercialized will largely depend on the market needs and patient benefits of these new products, whereby the regulatory burden will play a major role. In this light, promising applications in the area of tissue engineering will definitely not be less challenging than those faced so far with the classical therapeutics.

CONCLUSIONS

Since the initial discovery of coacervation in the 1930s, a very large number of studies have dealt with coacervation, the underlying mechanisms, thermodynamics, materials, and applications. Nonetheless, relatively modest success and innovation in coacervation-based pharmaceutical systems have emerged. Clearly, the scientific achievements in this field, for example for the treatment of diabetes or the design of bioartificial organs, outweigh so far the commercial usefulness and benefit. Recently, however, coacervation has attracted great interest in the areas of DNA delivery from DNA-polymer complexes, in biotechnology, and in the food and agricultural fields. In the food sector, flavoring agents or agents with undesirable flavors, odors, acids, bases, artificial sweeteners, colorants, preservatives, leavening agents, among others, have been encapsulated by coacervation into materials such as starches, dextrins, alginates, proteins, and lipids. In the agricultural field, coacervation-based microencapsulation is used, e.g., for coating seeds. Finally, the ultimate relevance of coacervation may be in the biological existence and function of intracellular and extracellular complexes such as heparin-protein, DNA-protein, adrenergic agonists-protein. The association and dissociation of these complexes are very sensitive to pH, inorganic ions and electrical potential at membranes. These characteristics suit a plausible, though not elucidated process by which some pharmacological events may occur.

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